ABSTRACT

A recombinant gene encoding a single-chain variable fragment (scFv) antibody against Venezuelan equine encephalitis virus (VEE) being cloned into a prokaryotic T7 RNA polymerase-regulated expression vector was disclosed. A streptavidin-binding peptide (SBP) sequence fused to a 6His tag is then attached downstream to the scFv gene. The recombinant fusion protein is expressed in bacteria and then purified by immobilized metal affinity chromatography. ELISA and Western blotting results revealed that the fusion protein not only retained VEE antigen binding and specificity properties similar to those of its parent native monoclonal antibody, but also possessed streptavidin-binding activity. This discovery obviates the need for chemical biotinylation of antibodies and the risk associated with antibody denaturation and provides a stable and reproducible reagent for rapid and efficient immunoassay of VEE.